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Short communication

The determination of levofloxacin by flow injection analysis using UV detection, potentiometry, and conductometry in pharmaceutical preparations

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Abstract

A flow injection analysis (FIA) using UV detection, potentiometry and conductometry for levofloxacin (LVF) are described in this study. The best solvent system was found to consist of 0.2 M acetate buffer at pH 3 having 10% MeOH. A flow rate of 1 ml min^{-1} was pumped and active material was detected at 288 nm. The detection limit (LOD) and limit of quantification (LOQ) for FIA were calculated to be 3×10^{-7} M (S/N = 3) and 1×10^{-7} M (S/N = 10), respectively. In the analysis of tablets, the RSD values were found to be 0.83, 0.98 and 0.99 for FIA, potentiometric and conductometric methods, respectively.

Keywords: Levofloxacin; Flow injection analysis; Potentiometry; Conductometry; Spectrophotometry; Pharmaceutical applications

1. Introduction

Levofloxacin (LVF), (−)-(S)-9-fluoro-2.3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxilic acid hemihydrate, is a quinolone antimicrobial agent which exhibits broad-spectrum in vitro bactericidal activities against gram-positive and gram-negative aerobes. LVF is the pure (−)-(S)-enantiomer of the racemic drug substance ofloxacin [1]. It is chemical structure is demonstrated in Fig. 1.

A number of studies have been reported for the determination of LVF including synchronization-first-derivative fluorescence spectroscopy [2], spectrofluorimetric [3], terbium-sensitised luminescence [4] and HPLC [5–9].

Flow injection analysis (FIA) is a new methodology characterised by its versatility, ease of automation, high sampling frequency and minimum sample treatment prior to injection into the system. The FIA techniques have found wide applications recently, mainly due to reduction of the analysis time and reagents consumption compared to conventional manual procedures [10]. On the other hand, their high sensitivity makes them suitable for the determination of low concentra-

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tions of pharmaceuticals in biological fluids when used as detectors in HPLC. They can also optimise the detection of analyse independently from the way process occurring in the chromatographic column [11]. In addition; potentiometric and conductometric titrations were suitable for the determination of the relatively large amount of the drugs. The apparatus required for making potential and conductance measurements and performing titrations are generally inexpensive and basically simple in details. For this reason, the measurements of potential (or pH) and conductance finds wide acceptance in industry as an analytical tool, both in the laboratory and in the process and quality control for routine analyses [12,13]. The aim of this study is the direct determination of LVF by FIA, potentiometric and conductometric methods and the application to the pharmaceutical preparations.

2. Experimental

2.1. Apparatus and chemicals

WTW Multiline P4 Universal potentiometer-conductometer cabled WTW Sen-Tix 97T combined glass pH electrode and WTW Tetracon 325 conductometric electrode cell (Germany), a Shimadzu Spectrophotometer Model UV 2401 PC (Japan) and quartz cells in the measurement of the absorbance were used.

The HPLC apparatus used a Model LC 6A pump equipped with a 20 μl manual loop injector, a Model SPD-A10 UV variable wavelength detector and a Model C-R7A integrator (all Shimadzu, Japan).

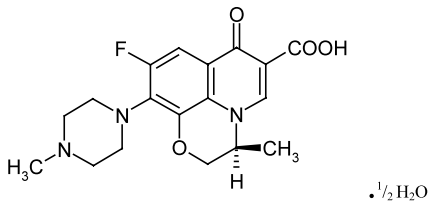

Fig. 1. The chemical structure of LVF.

Standard LVF (hemihydrate, 99.8%) and tablets (Cravit®) containing 500 mg active material were kindly supplied from Fako Ilaclari A.S. (Istanbul, Turkey). Other chemicals were of analytical grade of E.Merck.

2.2. Procedures

2.2.1. Flow injection analysis

A stock solution of LVF (1×10^{-3} M) was prepared using bidistilled water and the dilutions were made in the range of 1×10^{-6}–5×10^{-6} M. As the carrier phase an aqueous solutions of MeOH (10%, v/v) was used. The buffer solutions were prepared using 1 M CH_3COONa (pH 1–6) and 1 M K_2HPO_4 (pH 7–11) and their pH values were adjusted in the range of 1 and 11 using 2 M HCl or 2 M KOH.

2.2.2. Potentiometry and conductometry

Standard LVF was weighed, transferred to a beaker, added 30 ml ethanol and titrated by 0.1 M NaOH. Buffer solutions of pH 4.87 and 8.05 for pH-meter, 0.01 M KCl for conductometer were used in the calibration. Both electrode submerged into the titration solution, potential and conductivity were recorded at the same time of the addition of each titrant volume.

2.2.3. Spectrophotometry

A series of standard LVF dilutions in the concentration range 1×10^{-5} and 5×10^{-5} M was prepared using 1×10^{-3} M stock solution. As the solvent of LVF 0.1 M NaOH was employed. Calibration equation was calculated measuring the absorbance values of the standard solutions at 288 nm.

2.3. Application to the tablets

Twenty tablets were weighed and finely powdered in a mortar. The average weight of a tablet was calculated. For the FIA, a sample equivalent to one tablet was weighed and transferred to a 100 ml calibrated flask, 20 ml acetate buffer (1 M, pH 3) was added, magnetically stirred for 20 min and made up to volume with bidistilled water. A sufficient amount of the solution was pipetted in a

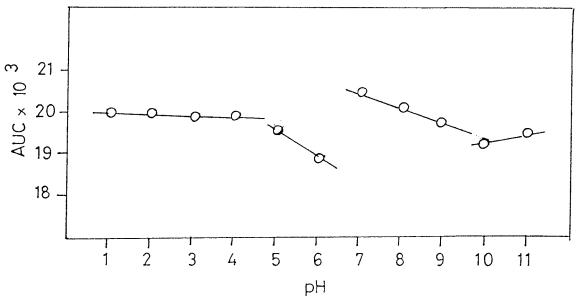

Fig. 2. Variation in the AUC values of LVF (1×10^{-6} M) in relation to pH.

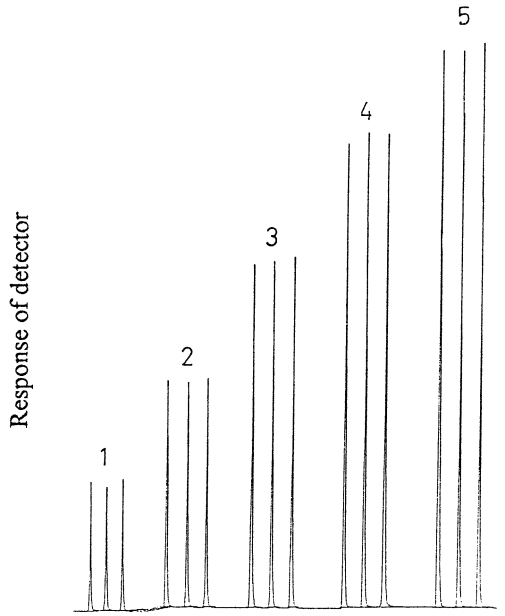

Fig. 3. The signals in the 1×10^{-6}–5×10^{-6} M concentration range of LVF with three replicate injections.

tube and it was centrifuged for 10 min. The supernatant was diluted to the predetermined values and injected in to sample loop by means of a syringe.

For potentiometry and conductometry, the powder of the tablets equivalent to an average tablet was weighed, transferred to a beaker, added 30 ml ethanol and titrated by standard NaOH.

Table 1
Linearity and accuracy of FIA method for LVF

Parameters	Intra-day precision ($k = 1$; $n = 8$)	Inter-day precision ($k = 4$; $n = 32$)
Slope ± S.D.	$1.81 \times 10^{10} \pm 369$	$1.77 \times 10^{10} \pm 489$
Intercept	3656	3678
Correlation coefficient (r)	0.9996	0.9992
Slope ± CL ($P = 0.05$)	$1.81 \times 10^{10} \pm 474$	$1.79 \times 10^{10} \pm 571$

S.D., standard deviation; CL, confidence limit; k, number of the set; n, number of the sample.

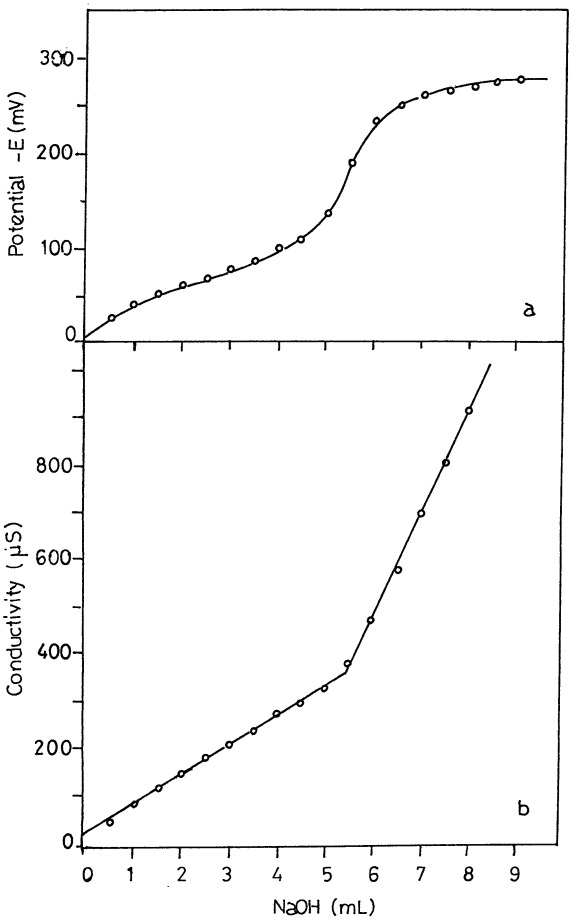

Fig. 4. Potentiometric(a) and conductometric (b) curves for the titration of LVF (500 mg) with 0.1 N NaOH.

Table 2
Assay results of LVF in tablets*

	FIA	Potentiometry	Conductometry	UV
Mean	494	492	490	496
n	8	8	8	8
RSD%	0.83	0.98	0.99	0.87
Confidence limit ($P = 0.05$)	± 1.86	± 1.14	± 2.17	± 1.92
t-test of significance	1.69	1.81	1.96	$t_{0.05} = 2.14$ (table)
F-test of significance	1.11	1.25	1.29	$F_{0.05} = 4.17$ (table)

* Each tablet contains 500 mg of LVF.

3. Results and discussion

To determine the parameters for the optimisation, an LVF solution having 5×10^{-6} M was used. The solvent system consisted of MeOH and bidistilled water. To investigate the percentage of MeOH, it was varied beginning from 10 to 50% (v/v). It was found that the optimum concentration of MeOH, in view of peak morphology, was 10% (v/v). To determine the optimum flow rate; the flow rate was changed from 0.5 to 3 ml min^{-1} and the best flow rate was found to be 1 ml min^{-1}. The final concentration of buffer in the test solutions was 0.2 M. When the base line was reached, another sample was injected. The peak areas versus pH are illustrated in Fig. 2.

As seen in Fig. 2, the peak areas showed significant differences above pH 5. This variation can be attributed to the zwitterionic formation of molecule in the range of pH 5–10. This pH value corresponded with the approximate pK_{a_1} and pK_{a_2} values of LVF, respectively. The pK_{a_1} and pK_{a_2} values of norfloxacin that are structurally related to LVF have been reported 5.50 ± 0.17 and 9.67 ± 0.21, respectively. [14]. These data have supported the approach mentioned above. However, these differences were minimum at pH values between 1 and 5. Therefore, the acetate buffer of pH 3 was chosen as working pH. The signals for the LVF at concentrations ranging from 1×10^{-6} to 5×10^{-6} M were obtained under the conditions described above and they are given in Fig. 3.

Although the prepared solutions give the same signals during a week, it is not always possible to obtain the true stability of the molecule. For this aim, the HPLC and TLC method are recommended.

The relationship between area under curve (AUC) against LVF concentration was found to be $AUC = 1.82 \times 10^{10} C\ (M) + 3696.9$, $r = 0.9997$. The detection limit (LOD) and limit of quantification (LOQ) were calculated to be 3×10^{-7} M (S/N = 3) and 1×10^{-7} M (S/N = 10), respectively.

Linearity and accuracy in the concentration range of 1×10^{-6}–5×10^{-6} M were examined employing intra-day and inter-day studies for the determination of LVF. The results were evaluated statistically and these are demonstrated in Table 1.

The titrimetric experiments were realised by submerging the combined glass pH electrode and conductometric cell into the same test solution. After addition of each titrant volume, the variations in the potential and the conductivity were recorded. Plotting the potential and conductivity versus the addition of titrant volume, a well-defined S-shape potentiometric and a good conductometric graph were obtained. Both graphs are illustrated in Fig. 4. At the beginning of titration, the solution of LVF was turbid but its transparency increased gradually around the equivalence point. The equivalence points of LVF were calculated using second-derivative curve and the intersection point for the potentiometric and conductometric methods, respectively.

3.1. Application to the pharmaceutical dosage forms

The proposed technique was applied to the

tablets. The ingredients in the tablets did not interfere in the experiments. The AUC was used for calibration. Spectrophotometry was chosen as a comparison method for the determination of LVF. The absorbance of LVF in 0.1 M NaOH solution was measured at 288 nm. The relationship between absorbance (A) and concentration (C) was found to be: $A = 23408C$ (M) $+ 0.009$; $r = 0.9999$.

The determination methods progressed in the study were applied to the pharmaceutical dosage forms and the results are tabulated in Table 2.

It was observed that the differences among the methods are insignificant at the 95% probability level (F- and t-test). As a conclusion, the methods proposed in this study are simple, accurate, precise and rapid. Therefore, the suggested methods can be used in the pharmaceutical dosage forms for routine analysis of LVF.

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